

Usefulness of lymphoid granulomatous inflammation culture obtained by endobronchial ultrasound-guided transbronchial needle aspiration in a fungal endemic area

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ABSTRACT

Background and Objectives: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is the procedure of choice for the evaluation of mediastinal/hilar lymph node enlargements. Granulomatous inflammation of the mediastinal/hilar lymph nodes is often identified on routine histology. In addition, mediastinal lymphadenopathy may be present with undiagnosed infection. We sought to determine the usefulness of routine cultures and histology for infectious etiologies in a fungal endemic area when granulomatous inflammation is identified. **Materials and Methods:** We identified 56 of 210 patients with granulomatous inflammation on EBUS-TBNA biopsies from October 2012 through October 2014. An onsite cytologist evaluated all biopsies and an additional TBNA pass for microbiologic stains and cultures were obtained in those with granulomatous inflammation. **Results:** Of the 56 patients with granulomatous inflammation, 20 patients had caseating (necrotizing) granulomas while noncaseating (nonnecrotizing) granulomas were detected in 36 of the remainder patients. In patients with caseating granulomas, fungal elements were identified in 6 of 20 (30%) patients (histoplasma; $N = 5$, blastomyces; $N = 1$) on Grocott methenamine silver (GMS) stain. Lymph node cultures identified 3 of 20 (20%) patients as being positive for *Mycobacterium tuberculosis* ($N = 1$), *Histoplasma capsulatum* ($N = 1$), and *Blastomyces dermatitidis* ($N = 1$). Among patients with noncaseating granulomas, only 2 out of 36 (5%) were positive for fungal elements on GMS stain, identified as *Histoplasma*, although the lymph node cultures remained negative. **Conclusion:** The incidence of granulomatous inflammation of mediastinal lymph nodes was 26.6% in our series. Of these patients, noncaseating granulomas were more common (64% vs. 36%). Infectious organisms, fungal or acid-fast bacilli (AFB), on either staining or lymph node culture were rarely identified in noncaseating granulomas, 5% and none, respectively. Caseating granulomas were more commonly associated with positive lymph node fungal stain and culture, 35% and 15%, respectively. In a fungal endemic area, lymph node staining and culture can be considered in cases with caseating granulomatous inflammation, if known at the time of biopsy.

Key words: Culture, endobronchial ultrasound (EBUS), fungal, lymph node biopsy

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INTRODUCTION

Mediastinal lymphadenopathy is a nonspecific yet common finding on chest computed tomography (CT) scan, and it can be associated with a wide variety of pathologies including malignancy, infection, and inflammation. Traditionally, the mediastinal lymph nodes could only be sampled by mediastinoscopy, thoracic surgery, and transthoracic needle biopsy. With the advancement of endoscopic ultrasound (EUS) technology, lymph node biopsy can now be done real time during endoscopy and bronchoscopy, including endobronchial ultrasound (EBUS). The samples collected via EBUS can be utilized for rapid onsite evaluation, cytology, cell block, flow cytometry and culture.^[1]

EBUS-guided transbronchial needle aspiration (EBUS-TBNA) has become the procedure of choice for the evaluation of mediastinal/hilar lymph node enlargements in both benign and malignant diseases. EBUS-TBNA can be used to diagnose and stage lung cancer with less morbidity.^[2,3] In addition, this is done with high sensitivity (91%-95%) and specificity (100%). The prototypical inflammatory disease involving mediastinal lymph nodes is sarcoidosis. EBUS-TBNA has similar to better efficacy in diagnosing granulomatous disease consistent with sarcoid compared to conventional bronchoscopy.^[4]

Finally, the utility of EBUS sampling has not been well studied in infectious etiologies. EBUS-TBNA culture has not been demonstrated to increase diagnostic yield, even in tuberculosis endemic areas. EBUS-TBNA histology was found to be diagnostic for or suggestive of tuberculosis in 86% of the patients, while the EBUS-TBNA culture was positive in only 47% of the patients.^[5-9]

Information about the yield of EBUS-TBNA culture in fungal infection is scarce and limited to small case series. Besides isolated case reports showing positive EBUS-TBNA cultures for *Cryptococcus neoformans*, the cultures are reported to be negative in patients with known histoplasmosis.^[10-13]

There are currently no robust data or guidelines when or in what clinical scenario to send culture on EBUS-TBNA samples. However, granulomatous inflammation of the mediastinal/hilar lymph nodes is often identified on routine histology. In addition, mediastinal lymphadenopathy may be present with

undiagnosed infection. We aimed to determine the usefulness of cultures and histology of EBUS-TBNA samples for infectious etiologies in a fungal endemic area when granulomatous inflammation is identified.

MATERIALS AND METHODS

Patients

We retrospectively reviewed 210 consecutive patients who underwent EBUS-TBNA for the work-up of mediastinal or hilar lymph node(s) enlargement(s) during the period of October 2012 to October 2014. The study was approved by the Institutional Review Board. At least one enlarged mediastinal or hilar lymphadenopathy (short axis greater than 1 cm) was observed in all patients on CT chest images.

Serum fungal serology, urinary histoplasma antigen, and CT chest findings

Besides obtaining EBUS-TBNA culture and cytopathological data on each patient with granulomatous inflammation ($N = 56$), we also collected information on CT scan findings in those with fungal infection or tuberculosis confirmed or suggested by culture or cytological examination, serum fungal serology, and urinary histoplasma antigen, if performed.

EBUS-TBNA procedure

An interventional pulmonologist or a thoracic surgeon performed all procedures. All procedures are performed either in endoscopy unit with moderate sedation or in the operation room (OR) under general anesthesia. We uniformly started bronchoscopies with airway examination by using a flexible bronchoscope (Evis Exera, BF-MP160F or BF-H190, Olympus, Tokyo, Japan) followed by EBUS examination (BF-UC180F convex probe EBUS ultrasound bronchoscope powered by Aloka ProSound F75 ultrasound processor, Olympus, Tokyo, Japan). A dedicated 22-gauge needle (NA-201SX-4022, Olympus) is used to perform transbronchial aspiration.

Sample collection

We routinely perform 3-5 TBNA passes from each pathologically enlarged lymph node stations for smear and cellblock as well as an extra pass for stains [Grocott methenamine silver (GMS) and Ziehl-Neelsen stains] and cultures for acid-fast bacilli (AFB) and fungus when rapid cytological evaluation determines granulomatous inflammation. The extra pass is collected in a sterile culture container by instilling 2 mL of sterile

saline through the EBUS needle. First 1 or 2 passes of TBNA samples are smeared on a glass slide by instilling forced air through the EBUS needle. Then the aspirate on glass was stained with hematoxylin and eosin by a cytology technician while the rest of the passes were kept in formalin for cellblock preparation.

Onsite rapid cytologic examination

A cytopathologist was available either physically in the procedure room or via telecytology during all procedures. An immediate feedback was provided as soon as the slides are stained. Fifty-six out of 210 patients were found to have granulomatous inflammation on rapid evaluation. Although definitive determination of caseating versus noncaseating granulomas was reported 2-3 days later, onsite evaluation was suggested caseating granulomas in 10 out of 20 patients who were confirmed to have caseating granulomas on the final report.

RESULTS

Patient characteristics

We identified 56 patients with granulomatous inflammation on EBUS-TBNA biopsies. Demographic characteristics; underlying cancer diagnosis, if existed; and ongoing chemotherapy are shown in Table 1. Twenty-four out of 56 patients had a recent or remote history of thoracic or extrathoracic malignancy. Only 4 patients were receiving chemotherapy at the time of EBUS procedure.

Cytopathological evaluation and granuloma detection

All 56 patients found to have granulomatous inflammation by rapid onsite cytology. Of the 56 granulomas identified, 20 were determined as caseating, while 36 were determined as noncaseating granulomas. None of these patients had cytological evidence of malignancy.

TBNA and bronchoalveolar lavage stains and cultures

Twenty-two out of 56 patients with granulomatous inflammation had positive TBNA cultures; 1 for *Mycobacterium tuberculosis* (MTB), 1 for *Blastomyces dermatitidis*, 1 for *Histoplasma capsulatum* and the remaining cultures were positive for Streptococcal species and diphtheroids likely representing contamination. The patients with positive MTB or fungal cultures had caseating granulomas. Although only three of the TBNA cultures led to a diagnosis, TBNA fungal GMS stain and AFB stain came back

positive in seven patients including the ones with positive culture.

Two of the patients with noncaseating granulomas stained positive for histoplasmosis on TBNA, none grew on culture.

Bronchoalveolar lavage (BAL) cultures of patients with positive TBNA cultures for MTB and *B. dermatitidis* were also positive [Table 2].

Chest CT scan findings

Majority of the patients with granulomatous inflammation (46 of 56) had only mediastinal and/or hilar lymph node enlargement while 10 patients had nodule(s) and/or consolidation (greater than 1 cm) on CT chest [Table 2].

Serum and urine fungal serology

Serum fungal antibody and urine histoplasma antigen were tested in some of the patients [Table 2].

DISCUSSION

In this study, we examined the usefulness of EBUS-TBNA stain and cultures in 210 consecutive patients in a fungal endemic area. Of 210 patients, 56 were found to have granulomatous inflammation, either noncaseating (64%) or caseating (36%). We found infectious organisms, fungal or AFB, more frequently in both staining and cultures in patients with caseating granulomas (fungal infection in 35% and AFB in 15%) while none in patients with noncaseating granulomas. Twenty-two out of 56 patients with granulomatous inflammation had positive TBNA cultures; majority (86%, 19 of 22) were bacterial. Bacterial cultures were

Table 1. Patient demographics and underlying cancer diagnosis

Characteristic	Total number (median)
Sex	
Male	30 (53.5%)
Female	26 (46.4%)
Age	24-79 (54)
History of cancer	24 (42.8%)
Breast	8
Leukemia/lymphoma	6
Lung	3
Colon	3
Cervix/uterus	2
Thyroid	1
Larynx	1
Ongoing chemotherapy	4

Table 2. Results of diagnostic tests

Patient	TBNA cytology (granuloma)	TBNA GMS stain	TBNA fungal culture	Serum fungal antibody	Urine histoplasma antigen	TBNA AFB stain/culture	CT chest nodule/consolidation >1 cm	BAL AFB and KOH stain/culture	Final diagnosis
1	Caseating	Negative	Negative	Negative	N/A	+/MTB	Yes	AFB +/-MTB	MTB
2	Caseating	Small budding yeast	Negative	N/A	N/A	Negative	No	N/A	Histoplasmosis
3	Caseating	Small budding yeast	Negative	N/A	N/A	Negative	No	N/A	Histoplasmosis
4	Caseating	Small budding yeast	Negative	NA	N/A	Negative	No	N/A	Histoplasmosis
5	Caseating	Negative	Negative	Histoplasma	N/A	Negative	No	N/A	Histoplasmosis
6	Caseating	Broad based budding yeast	Blastomyces dermatidis	Negative	N/A	Negative	Yes	Yeast +/- blastomyces	Blastomycosis
7	Caseating	Small budding yeast	Histoplasma	Histoplasma	N/A	Negative	Yes	Negative/negative	Histoplasmosis
8	Non-caseating	Small budding yeast	Negative	Negative	N/A	Negative	Yes	Negative/negative	Histoplasmosis
9	Non-caseating	Small budding yeast	Negative	Negative	Negative	Negative	Yes	Negative/negative	Histoplasmosis

positive for Streptococcus and Diphtheroid species that are considered as contaminant. Ultimately, we diagnosed 8 of 56 (14%) patients with fungal or MTB infections.

Although our institution is not located at a MTB prevalent area and our only patient with tuberculosis was an immigrant from the Middle East; Minnesota being in the Mississippi Valley region is an endemic area for histoplasmosis.^[14,15] It is also estimated that greater than 80% of the adult population living in endemic areas were previously infected with histoplasmosis.^[16] Fungal infections are diagnosed based on demonstration of fungal elements on stains and cultures with addition of serum fungal serology and urine enzyme immunoassay for *Histoplasma* antigen.^[17,18]

The use of EBUS-TBNA cultures in infectious etiologies is not well studied and limited to case series. Currently, there are no guidelines or recommendations on the utility of TBNA stains or cultures on patients with suspected infections or those with granulomatous inflammation. Previously published case series reported insensitivity of TBNA cultures in the identification of *Histoplasma* infection.^[12,19]

Although these reports did not have any positive TBNA cultures, 2 out of 56 patients with granulomatous inflammation in our cohort cultured positive for histoplasmosis ($N = 1$) and blastomycosis ($N = 1$). Both the patients also had positive results on GMS stains of aspirates. BAL of blastomycosis case was positive on potassium hydroxide (KOH) staining and fungal culture. None of our patients were immunocompromised at the time of EBUS procedure.

Yet, we had two positive TBNA cultures for fungal infections; having positive stains in same patients hampers the necessity of cultures. It is, however, difficult to draw a conclusion from the limited number of patients. Although there is no evidence, we performed an extra TBNA pass in all our patients who had granulomatous inflammation on rapid onsite examination to determine the presence or absence of underlying fungal infection. Our TBNA stain and culture results indicated that the patients with caseating granulomas had more positive results.

We performed BAL in patients who had positive parenchymal findings, any nodule or consolidation greater than 1 cm, in addition to EBUS lymph node sampling. Besides the patients with MTB and blastomycosis, none of the seven histoplasmosis patients had positive KOH stain or cultures. Noncaseating granulomas can be manifestation of sarcoidosis or seen as sarcoid-like reaction in patients with cancer while caseating granulomatous inflammation represents an infectious etiology.^[20-22]

Two of our patients with noncaseating granulomas stained positive for histoplasmosis and this is very well related to previous histoplasmosis infection.

This study has limitations. First, this is a retrospective study and we did not have complete work-up, such as fungus serology, serum and urine, in all patients. Second, there is also selection bias of obtaining biopsies of only those who had granulomatous inflammation on site evaluation. We noted that there were other patients with granulomatous inflammation on final pathology

report whom we did not send for TBNA stains and cultures. Third, this study was conducted in a fungal endemic area, therefore, it may not represent the general population.

CONCLUSION

The role of EBUS-TBNA in the diagnosis of malignant and benign conditions, such as sarcoidosis, is well accepted. We demonstrated that caseating granulomas were more commonly associated with positive lymph node fungal stain and culture, 35% and 15%, respectively.

Although previous studies reported that EBUS-TBNA stains and cultures have limited value in infectious etiology of mediastinal/hilar lymph node enlargements, our study suggests the potential value of EBUS-TBNA sampling in the patients with caseating granulomas if the information is available during the bronchoscopy.

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Conflicts of interest

There are no conflicts of interest.

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